

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Tsien, et al. Art Unit: Unassigned  
Application No.: Unassigned Examiner: Unassigned  
Filed: February 5, 2002  
Title: LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS

BOX PATENT APPLICATION  
Commissioner for Patents  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

In advance of the first examination in this case, Applicants request amendment of the subject application, as follows:

IN THE CLAIMS

Please cancel claim 1 without prejudice.

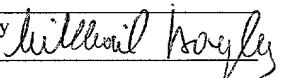
Please add the following claims 2-21:

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Mikhail Bayley



2. (New) A functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, and a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V or S65I, and wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein.
3. (New) The protein of claim 2, wherein the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.
4. (New) The protein of claim 2, wherein the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.
5. (New) The protein of claim 2, wherein the amino acid sequence further comprises a mutation at a position selected from the group consisting of Y145, N146, H148, M153, and V163.
6. (New) The protein of claim 2, wherein the amino acid sequence further comprises a folding mutation selected from the group consisting of F64L, V68L and S72A.
7. (New) The protein of any one of claims 2 to 6, which is a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

8. (New) A method of identifying a test chemical, comprising,
- 1) providing a sample containing a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by
    - i. at least the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, and
    - ii. a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V or S65I, andwherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein;
  - 2) contacting the sample containing the functional engineered fluorescent protein with the test chemical;
  - 3) monitoring the fluorescence of the sample in the presence of the test chemical compared to the fluorescence of the sample in the absence of the test chemical to determine whether the test chemical is active.
9. (New) The method of claim 8, wherein the amino acid sequence of the engineered fluorescent protein differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.
10. (New) The method of claim 8, wherein the amino acid sequence of the engineered fluorescent protein further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

11. (New) The method of claim 8, wherein the amino acid sequence of the engineered fluorescent protein further comprises a mutation at a position selected from the group consisting of Y145, N146, H148, M153, and V163.

12. (New) The method of claim 8, wherein the amino acid sequence of the engineered fluorescent protein further comprises a folding mutation selected from the group consisting of F64L, V68L and S72A.

13. (New) The method of claim 8, wherein the engineered fluorescent protein is a fusion comprising a polypeptide of interest and the functional engineered fluorescent protein.

14. (New) A method for determining whether a sample contains a target, comprising the steps of,

1) contacting the sample with a probe coupled to a functional engineered fluorescent protein, the functional engineered fluorescent protein comprising an amino acid sequence which is substantially identical to the amino acid sequence of *Aequorea* green fluorescent protein (SEQ. ID. NO:2) and which differs from SEQ. ID. NO:2 by

- i. at least the substitution T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, and
- ii. a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V

or S65I, and wherein said functional engineered fluorescent protein has a different fluorescent property than *Aequorea* green fluorescent protein;

2) determining whether the target has bound to the probe.

15. (New) The method of claim 14, wherein determining whether the target has bound to the probe comprises capturing the target on a solid matrix.

16. (New) The method of claim 14, wherein the amino acid sequence of the engineered fluorescent protein differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W.

17. (New) The method of claim 14, wherein the amino acid sequence of the engineered fluorescent protein further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W.

18. (New) The method of claim 14, wherein the amino acid sequence of the engineered fluorescent protein further comprises a mutation at a position selected from the group consisting of Y145, N146, H148, M153, and V163.

19. (New) The method of claim 14, wherein the amino acid sequence of the engineered fluorescent protein further comprises a folding mutation selected from the group consisting of F64L, V68L and S72A.

20. (New) The method of claim 14, wherein the probe is an antibody.

21. (New) The method of claim 14, wherein the probe is a receptor.

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PATENT  
Attorney Docket No.: REGEN1250-6

REMARKS

Claim 1 has been canceled and Claims 2-21 have been added.

This Preliminary Amendment is submitted in advance of the first examination of the subject application. No new matter has been entered.

Respectfully submitted,

Date:

2/5/02

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